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Applicant : BIO-K PLUS INTERNATIONAL INC. *ET AL.*
International application No. PCT/CA2004/001968
International filing date : 15 November 2004
Priority date : 13 November 2003
Title : USE OF STRAIN OF BACILLUS AND BYPRODUCTS THEREOF FOR INHIBITING FORMATION OF BLOOD VESSELS
Agent's file reference : 000595-0052

AMENDMENT UNDER ARTICLE 34

Canadian Intellectual Property Office
Gatineau (Quebec) K1A OC9
CANADA

To the attention of Ms. Cynthia Brewer
Authorized officer

Madam :

In response to the Written Opinion of the International Searching Authority dated March 14th, 2005, please amend the above-identified application as follows.

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IN THE CLAIMS:

Please cancel pages 18 to 21 containing claims 1 to 31 presently on file and insert the corresponding pages containing new claims 1 to 30.

REMARKS

MODIFICATIONS TO THE CLAIMS :

In response to the Examiner's rejection of the claims for lack of novelty and inventive activity, claim 1 presently on file has been limited to a mixture of *Lactobacillus acidophilus, Lactobacillus casei* and their broth, and claim 24 presently on file has been limited by incorporation of the subject matter of claim 25 presently on file. Original claim 25 has thus been deleted. The numbering and dependence of subsequent claims have been corrected accordingly.

Response to the Examiner's rejection of the claims for lack of novelty in view of D1 to D3

The Applicant has noted the Examiner's rejection of claims 1 to 8, 14 to 26 (renumbered 25) and 31 (renumbered 30) for lack of novelty in view of D1 (WO 03/045405), of claims 1, 5 to 10, 12 to 15, 17 to 19, 21 to 24 and 26 (renumbered 25) for lack of novelty in view of D2 (Arimochi et al.) and of claims 1, 14, 15, 17, 19, 21 and 23 in view of D3 (Kato et al.)

The Applicant submits that newly submitted claims are now related to a lactic composition for the prevention or the treatment of angiogenesis dependant disorders comprising a mixture of at least a *Lactobacillus acidophilus* strain such as *Lactobacillus acidophilus* I-1492 and *Lactobacillus casei* and their broth.

They are also related to a supernatant obtained from the lactic composition of the invention as an antiangiogenic agent; to the use of the composition of the invention or the supernatant of the invention in the prevention or the treatment of angiogenesis dependant disorders in a mammal; a method for prevention or treatment of an angiogenesis dependant disorder by administering to a mammal the lactic composition or the supernatant of the invention and also to a method of obtaining the supernatant of the invention; whereby the lactic bacterial strains are suspended on a complex MRS medium.

D1 discloses the use of a lactic bacterial strain with the combination of an anti-cancer agent such as 5-fluoro-uracil to treat or prevent cancer, particularly colon cancer. D1 also discloses the use of the supernatant of the lactic bacterial strain in combination with 5-fluoro-uracil to study the apoptosis of cancer cell lines and more particularly, human colon cancer LS 513 cell lines.

D1 does not disclose the lactic bacterial composition comprising a mixture of lactic bacterial strains and their broth, its use as an antiangiogenic; the use of the supernatant of the present invention for treatment or prevention of angiogenesis dependant disorders, nor a method for prevention or treatment of angiogenesis dependant disorders by administering the composition according to the invention to a mammal.

Hence, it is clear from the above that D1 taken alone does not disclose the subject matter of newly submitted claims 1 to 8, 14 to 26 (renumbered 25) and 31 (renumbered 30).

D2 discloses the use of an animal model in the *in vivo* inhibition of precursor lesions of colon cancer by *Lactobacillus acidophilus* and its supernatant. D2 discloses *Lactobacillus acidophilus* strain ATCC 4356 only. Moreover, according to D2, the lactic bacterial strain is grown on degassed GAM broth.

D2 is also silent as to the effect of *Lactobacillus acidophilus* and the supernatant on angiogenesis and angiogenesis dependant disorders.

Hence, it is clear from the above that D2 taken alone does not disclose the matter of the newly submitted claims 1, 5 to 10, 12 to 15, 17 to 19, 21 to 24 and 26 (renumbered 25).

D3 discloses the ability of *Lactobacillus casei* to reduce the incidence and development of type II collagen induced arthritis in mice which is an animal model of rheumatoid arthritis in humans. Moreover, the *Lactobacillus casei* strain Shirota disclosed in D3 is in solid form and had been re-suspended in distilled water and had not been incubated in a complex medium before use as it is done in the present invention.

It is thus clear from the above that D3 taken alone does not disclose the subject matter of newly submitted claims 1, 14, 15, 17, 19, 21 and 23.

The Applicant thus submits that it is clear from the above arguments that none of D1, D2 or D3 taken alone discloses the subject matter of the newly submitted claims. Hence newly submitted claims 1 to 30 are new in view of the prior art.

In view of the above arguments, the Examiner is kindly requested to reconsider his rejection of the claims for lack of novelty in view of D1 to D3.

Response to the Examiner's rejection of claims 1 to 23 and 27 to 30 for lack of inventive step in view of D1, D2, D4 to D6

A.- The Applicant has noted the Examiner's rejection of claims 1 to 23 for lack of inventive step in view of D2 and D4 to D6.

In this regard the Applicant submits:

D4 teaches that the antitumorogenic activity of *Lactobacillus acidophilus* may be mediated by its direct antagonistic effect against a specific group of organisms. However, this document does not disclose nor teach the composition of the present invention, the activity of the composition of the invention or the supernatant of the invention as an antiangiogenic, as described by the newly submitted claims

Hence, a person skilled in the art, by reading D4 would not be led directly and without undue experimentation to the antiangiogenic composition of the invention nor to its use to prevent angiogenesis dependant disorders.

D5 teaches the use of the genus *Lactobacillus* to prevent a variety of diseases through modulation of the host immune system. D5 discloses the antitumor, antimetastatic and immunomodulatory activities of *Lactobacillus casei*, both *in vitro* and *in vivo*, but it does not disclose the composition of the invention containing a mixture of *Lactobacillus acidophilus, Lactobacillus casei* and their broth, as an antiangiogenic agent, not it discloses the use of the composition for the prevention or for the treatment of angiogenesis dependant disorders.

D6 is a review article and merely discloses the molecular pathways associated with physiological angiogenesis and reviews pathological angiogenesis, and the relationship of angiogenesis and cancer, diabetes retinopathy and arthritis.

First, the Applicant submits that none of D4 to D6 teaches the invention as claimed by newly submitted claims 1 to 30.

The Applicant also submits that a person skilled in the art by reading D2 in conjunction with D4 or D5 in combination with D6 would not be led directly and without undue experimentation to the composition or the use of the composition or the supernatant according to the invention since none of D2,

D4 or D5 teaches nor suggests the relationship between *Lactobacillus acidophilus* and *Lactobacillus casei* and their broth with angiogenesis, nor do they suggest or teach a method of preventing or treating angiogenesis dependant disorders by using *Lactobacillus acidophilus* and *Lactobacillus casei* and their broth.

In view of the above arguments, the Applicant submits that submitted claims 1 to 23 are inventive in view of the cited prior art.

B.- The Applicant has noted the Examiner's rejection of claims 27 to 30 presently on file (renumbered 26 to 29) for lack of inventive step in view of D1 and D2, and because obtaining a bacterial culture supernatant via centrifugation falls within the scope of routine laboratory practice.

In this regard the Applicant submits that claims 27 to 30 presently on file (renumbered 26 to 29) depend on claim 24 which has been considered inventive by the Examiner in view of D1 and D2. Hence they should also be considered inventive in view of D1 and D2.

In view of the above arguments, the Examiner is kindly requested to reconsider his rejection for lack of inventive step.

Response to the Examiner to other rejections

In response to the Examiner's rejections, the word "Description" appearing on page 1, line 9 has been corrected.

In response to the Examiner's rejection, the numbers of the Figures referred to on page 16, line 34 and page 17, line 3 have also been corrected.

In response to the Examiner's rejection of claims 1 to 4 because of the expression "is useful", this expression has been replaced by - - is for - -.

In view of the above Amendment and comments, the Applicant believes that the present application as amended now meets the requirements of the PCT and the invention as now claimed should be held patentable.

Respectfully submitted,

ROBIC
Patent Agents (No. 4078)

ZW/MA/hl/cl

Encls.: - New set of claims; and
- A/R card.

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21 18 Corrigé par l'OPIC SH 5/7/15

CLAIMS

1. A lactic composition comprising a mixture of bacterial strain, *Lactobacillus acidophilus and Lactobacillus casei*, and a whole broth of each of said mixture, characterized in that the lactic composition is for the prevention or the treatment of angiogenesis dependant disorders.

2. A lactic composition according to claim 1, characterized in that the at least one *Lactobacillus acidophilus* strain is strain I-1492 deposited at the CNCM.

3. A lactic composition according to claim 2, characterized in that it comprises at least 500 millions per gram of a population of living and active micro-organisms of the *Lactobacillus acidophilus* strains after 90 days under refrigeration, where at least 380 millions per gram are micro-organisms of the *Lactobacillus acidophilus* CNCM I-1492 strain.

4. A lactic composition according to claim 3, characterized in that it further comprises fermented milk proteins or fermented soy proteins.

5. A supernatant obtained from the lactic composition as defined in any one of claims 1 to 4, characterized in that said supernatant exhibits antiangiogenic properties.

6. The supernatant according to claim 5, characterized in that said supernatant is concentrated.

7. The supernatant according to claims 5 or 6, characterized in that said supernatant is 10X concentrated.

8. The supernatant according to any one of claims 5 to 7, characterized in that it comprises molecules of a size larger than 5000 kDa.

AMENDED CLAIMS

9. Use of the supernatant as defined in any one of claims 5 to 8, as an antiangiogenic agent.

10. Use of the supernatant as defined in any one of claims 5 to 8, in the prevention or the treatment of an angiogenesis dependant disorder in a mammal.

11. Use according to claim 10, wherein said mammal is a human being.

12. Use according to claim 10, wherein said disorder is selected from the group consisting of retinopathy, infantile haemangioma, rheumatoid arthritis, psoriasis, duodenal ulcers, post-angioplasty restenosis and tumour growth.

13. Use of a supernatant according to claim 12, wherein said disorder is tumour growth.

14. Use of the lactic composition as defined in any one of claims 1 to 4, as an antiangiogenic agent.

15. Use of the lactic composition as defined in any one of claims 1 to 4, in the prevention or the treatment of an angiogenesis dependant disorder in an mammal.

16. Use according to claim 15, wherein said mammal is a human being.

17. Use according to claim 15, wherein said disorder is selected from the group consisting of retinopathy, infantile haemangioma, rheumatoid arthritis, psoriasis, duodenal ulcers, post-angioplasty restenosis and tumour growth.

18. Use according to claim 17, wherein said disorder is tumour growth.

19. Method for prevention or treatment of an angiogenesis dependant disorder, the method comprising the step of administering to a mammal an effective

amount of the lactic composition as defined in anyone of claims 1 to 4 or of the supernatant as defined in any one of claims 5 to 8.

20. Method according to claim 19, wherein said mammal is a human being.

21. Method according to claim 19, wherein said disorder is selected from the group consisting of retinopathy, infantile haemangioma, rheumatoid arthritis, psoriasis, duodenal ulcers, post-angioplasty restenosis and tumour growth.

22. Method according to claim 21, wherein said disorder is tumour growth.

23. Method according to any one of claims 19 to 22, wherein said administration is oral administration.

24. A method of obtaining the supernatant as defined in any one of claims 5 to 8, characterized in that it comprises the steps of:
    a. suspension of at least one lactic acid bacteria strain selected from the group consisting of *Lactobacillus acidophilus* and *Lactobacillus casei* in a complex MRS medium to get a suspension;
    b. incubation of the suspension;
    c. dilution of the suspension in said suitable medium;
    d. incubation;
    e. centrifugation to obtain an liquid; and
    f. filtration said liquid to obtain the supernatant.

25. The method according to claim 24, characterized in that the incubation of step d is at 37°C.

26. The method according to claim 24, characterized in that the centrifugation is at 1000 x g for 15 min.

27. The method according to claim 24, characterized in that the filtration occurs on a 0.45 μm filter then on a 0.22 μm filter.

AMENDED CLAIMS

28. The method according to anyone of claims 24 to 27, characterized in that it further comprises the steps of:

    g-adding the supernatant of step f to Ultrafree-4™ tubes;

    h-centrifugation to obtain two layers;

    i-separation of the two layers into two separate Eppendorf™ tube.

29. The method according to claim 28, characterized in that centrifugation is at 3000 x g for 30 min.

30. The method according to any one of claims 24 to 29, wherein at least one *Lactobacillus acidophilus* strain is strain I-1492 deposited at the CNCM.